Tetrahedron Vol. 42, No. 9, pp. 2575 to 2586, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00
Pergamon Journals Ltd.

THE SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF (1S,2S)-1-HYDROXY-2-[(S)-VALYLAMINO]CYCLOBUTANE-1-ACETIC ACID (1) AND (1S,2S)-1-HYDROXY-2-AMINOCYCLOBUTANE-1-ACETIC ACID (2)

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(*Received in UK* 28 *February* 1986)

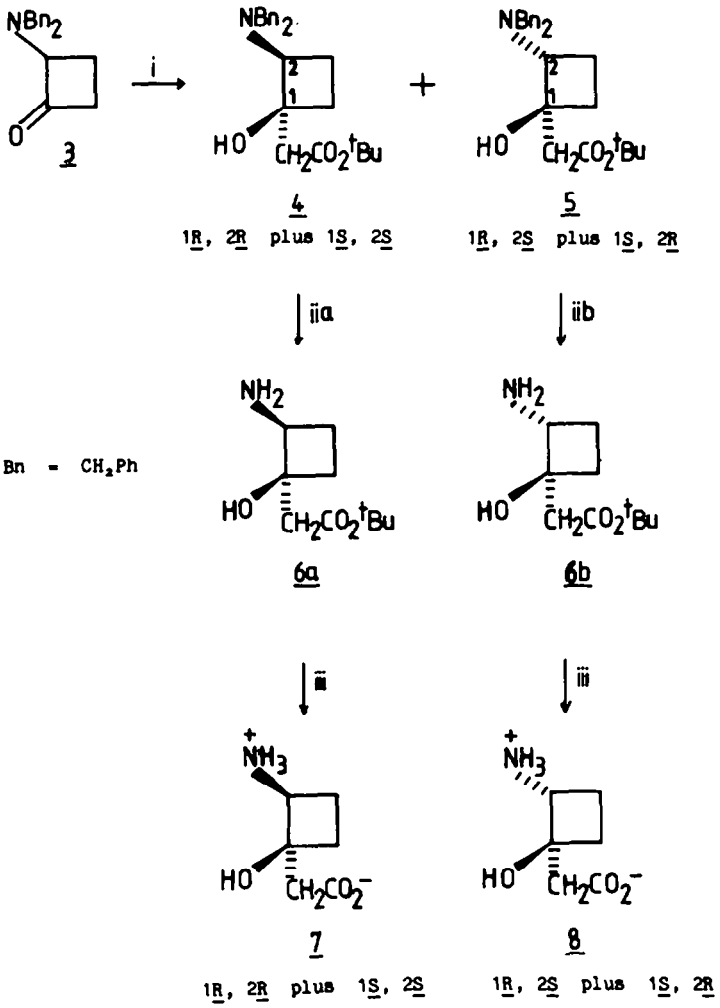
Abstract The unusual cyclobutanol containing dipeptide (1S,2S)-1-Hydroxy-2-[(S)-valylamino]cyclobutane-1-acetic acid (1), produced by an as yet unidentified Streptomyces species X-1092, has been synthesised via a short stereoselective route. Acid hydrolysis of (1) gave (1S,2S)-1-hydroxy-2-aminocyclobutane-1-acetic acid (2) which in direct comparison tests to (1) gave enhanced antibacterial activity against the gram-positive organism Bacillus subtilis A.T.C.C. 6633. An intriguing mechanism in which (2) acts as a "suicide substrate" for the crucial pyridoxal based enzyme cystathionine-γ-synthetase thus creating the antibacterial effect is proposed; a biomimetic study supports this proposal.

During a search for amino-acid antimetabolites in microorganisms, the cyclobutanol containing dipeptide (1) was isolated from an unidentified Streptomyces species X-1092 and shown to inhibit growth of both gram-positive[1a] and gram-negative[1b] organisms. This inhibition could be reversed by L-cysteine or L-cystine, and was partially reduced by L-methionine or D,L-homocysteine[1a]. Since this implies a possible interference by (1) of cysteine/methionine metabolism, and since the structure is unique thus far among microbial peptides, coupled with a relatively poor production (<4 mg l^{-1})[1a] we undertook to develop an efficient and stereoselective synthesis of this unusual compound.† We argued that the antibacterial effect of (1) may result from the known tendency for many peptide antibiotics to undergo a preliminary hydrolysis within the bacterial cell wall to release the active agent ("warhead hypothesis")[2]. In order to test this hypothesis, (1S,2S)-1-hydroxy-2-aminocyclobutane-1-acetic acid (2) was obtained chemically by acid hydrolysis of (1) and was shown to have more potent antibacterial activity than (1) against the gram-positive organism Bacillus subtilis.

Our synthetic strategy was based upon the expected trans selective addition‡ of the acetic moiety to an α-dibenzylaminocyclobutanone (Scheme 1). Thus 2-(dibenzylamino)cyclobutanone (3) [obtained in two steps from diethyl succinate][7] gave under Reformatsky conditions with t-butylbromoacetate, a mixture of racemic cis-(4) and racemic trans-dibenzylamino alcohols (5) [70%, (4):(5) = 8:1] which were readily separated by flash chromatography on silica gel. The configuration of the less polar trans-isomer (5) was confirmed by X-ray analysis of the crystals from acetone, m.p. 110-111°C . Debenzylation of (4) gave the racemic amino alcohol (6a) which upon treatment with TFA/anisole and subsequent purification by ion-exchange and H.P.L.C gave racemic (7) (75%). A similar sequence with (5) gave racemic (8) (78%).

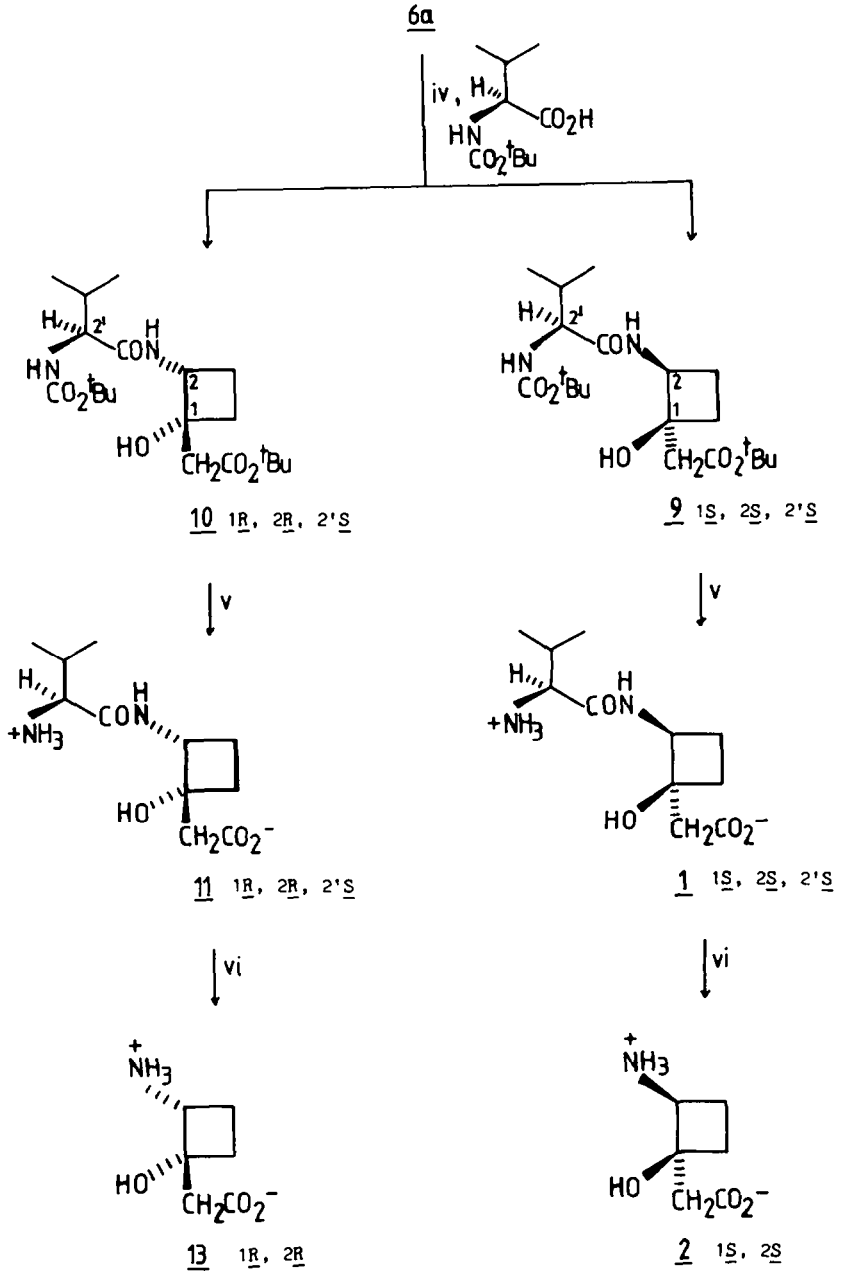
The crude amine (6a) was coupled to N-t-butoxycarbonyl-(S)-valine to yield a mixture of diastereoisomeric dipeptides (9) and (10) [69% from (4); ratio 1:1]. Recrystallization from diethyl ether gave pure 1S,2S,2'S-(9) m.p. 165-6°C; alternatively the two diastereoisomers (9) and (10) could be separated by H.P.L.C.

Deprotection of (9) with TFA/anisole followed by purification by ion-exchange and H.P.L.C. gave 1S,2S,2'S-(1), m.p. 239-246°C (Lit.[1a], 247-50°C),with identical ^1H and ^{13}C n.m.r. to an authentic sample.*


NBn2
i
3
4
5
1R, 2R plus 1S, 2S
1R, 2S plus 1S, 2R
iia
iib
Bn = CH2Ph
6a
6b
iii
iii
7
8
1R, 2R plus 1S, 2S
1R, 2S plus 1S, 2R

Scheme 1

Scheme 1 continued



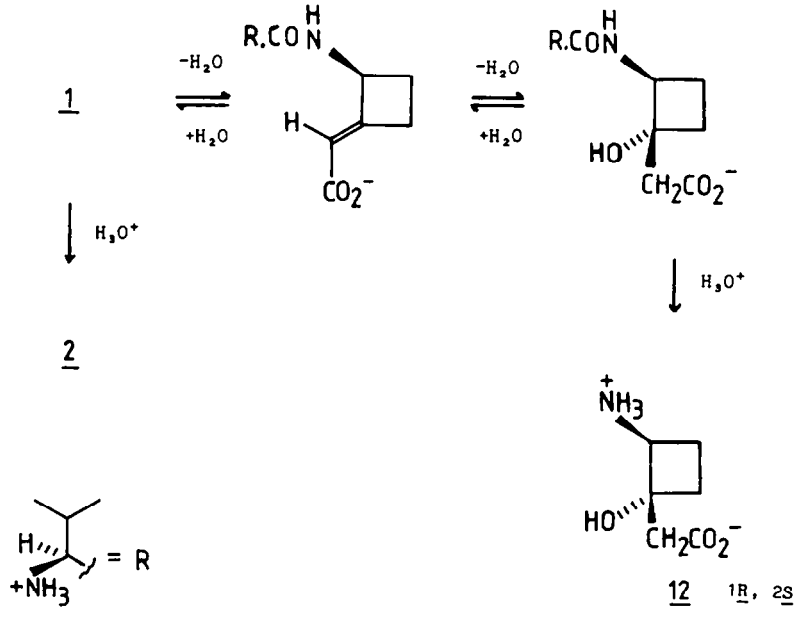
Reagents: (i) $BrCH_2CO_2{}^tBu$, Zn, THF, reflux, 90 min.; (iia) Pd on carbon, H_2 (1 atm.), MeOH, 27-30°C; (iib) Pd on carbon, H_2 (1 atm.), EtOH, 27-30°C; (iii) TFA, 20-25°C, 30 min; (iv) EEDQ, CH_2Cl_2, 24h.; (v) TFA, 20-25°C, 30 min; (vi) 2N HCl, reflux, 10-20h.

Scheme 1

Deprotection of the unseparated 1:1 mixture of (9) and (10) followed by purification by ion-exchange and H.P.L.C.‡ gave (1) (94%) and 1R,2R,2'S-(11) (90%), m.p. 201-210°C.

Acidic hydrolysis of 1S,2S,2'S-(1) [2M HCl, reflux, 10-20h.] gave a mixture containing 1S,2S-(2),1R,2S-(12) and unreacted (1), from which the desired amino-acid 1S,2S-(2) (10%), $[\alpha]^{2}\beta$ + 8.4° (c 0.13, H_2O) could be purified by preparative paper electrophoresis (at pH 1.8) and H.P.L.C. The production of 1R,2S-(12) during the hydrolysis (observed in the n.m.r. spectrum of the crude hydrolysis product) presumably results from an acid catalysed elimination/hydration process (e.g. Scheme 2). In a similar experiment, Scannell[1a] observed that acid hydrolysis of (1) gave S-valine and "varying ammounts of at least 3 other compounds which were not purified or characterised".

Acid hydrolysis of 1R,2R,2'S-(11) gave a mixture containing 1R,2R-(13), 1S,2R-(14) and (11) from which the 1R,2R-(13) isomer could be similarly isolated (20%), $[\alpha]^{2}\beta$ - 7.8° (c 0.11, H_2O).



Scheme 2

The antimicrobial activity of the synthetic and authentic[8] samples of (1) were identical when Staphylococcus aureus N.C.T.C. 6571, Bacillus subtilis A.T.C.C. 6633 and Escherischia coli ESS were the test organisms (Table 1). The antibacterial effects of (7), (8), (11), (1), (13), and (2) were also tested against both the gram-positive organism Bacillus subtilis A.T.C.C. 6633 (Table 2) and the gram-negative organism Escherischia coli ESS (Table 3). The results indicate that with gram-negative organisms, only the dipeptide (1) gave antibacterial activity. The dipeptide (1) is an antimicrobial agent which, with gram-negative organisms, presumably enters into the bacterial cell by the so-called "illicit transport" mechanism[2]. Thereafter hydrolysis releases the amino-acid (2) as the "warhead" within the cell wall which is responsible for the antibacterial action. The "illicit transport" mechanism required for gram-negative organisms is in agreement with the lack of antibacterial activity shown by the amino acid (2) with such organisms. Alternatively, with gram-positive organisms, (2) shows a greater antibacterial activity than (1) or the racemate (7) (at the same molar concentration). These results are in agreement with (2) and not (1) as the species responsible for the antibacterial action and highlight the necessity for the warhead (2) to be coupled to valine as (1), to be an effective antibacterial agent with gram negative organisms.

Test Organism	Synthetic substrate (1)/ μmol [a]			
	0.2	0.3	0.5	1.0
S.aureus N.C.T.C. 6571[b]	N/R	N/R	10	11
B.subtilis A.T.C.C. 6633[c]	18	24	N/R	N/R
E.coli ESS[c]	31	34	N/R	N/R
	Authentic substrate (1)*/ μmol[a]			
	0.2	0.3	0.5	1.0
S.aureus N.C.T.C. 6571[b]	N/R	N/R	10	11
B.subtilis A.T.C.C. 6633[c]	18	24	N/R	N/R
E.coli ESS[c]	31	34	N/R	N/R

a: Inhibition zone diameter (mm.) [hole size - 9.5mm] obtained from feeding substrate 0.2-1.0 μmol in water (100μl). N/R refers to no observation attempted.

b: A complex medium which was not entirely cysteine free was used for the growth of this organism. The plates contained 40μl of spores in 200ml of medium at pH 6.85-6.90 .

c: The growth medium for these organisms was cysteine free and asparagine (5%) was added as an additional nitrogen source. For B. subtilis 1ml of spores were mixed with 200ml of medium and for E. Coli 2ml of spores were mixed with 200ml of medium.

Table 1

Test Organism: Bacillus subtilis A.T.C.C. 6633

	Inhibition zone diameter (mm)					
Substrate for test/μmol[d]	(7)	(8)	(11)	(1)	(13)	(2)
0.1	15	-	-	11	-	29
0.2	24	-	-	18	-	37
0.3	32.5	-	-	24	-	40

d: Substrate applied in water (100μl).

Table 2

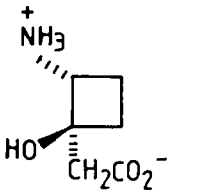
Test Organism: Escherichia coli ESS

	Inhibition zone diameter (mm)					
Substrate for test/μmol[e]	(7)	(8)	(11)	(1)	(13)	(2)
0.1	-	-	-	28.5	-	-
0.2	-	-	-	31	-	-
0.3	-	-	-	34	-	-

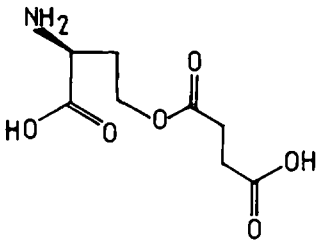
e: Substrate applied in water (100μl).

Table 3

A likely candidate for the site of inhibition by (2) is cystathionine-γ-synthetase, a crucial pyridoxal enzyme for methionine biosynthesis whose natural substrate, O-succinyl homoserine (15) bears at least a passing resemblence to (2). If indeed this were so, an intriguing "suicide" mechanism may be considered in which the Schiff base derivative of (2) and the pyridoxal co-factor might release the ring strain of the cyclobutanol, via a type of retro-aldol process, as in Scheme 3. Such a specific type of inhibition process which "shuts down" the in vivo synthesis of methionine (thus leading to bacterial death) can be reversed by addition of external L-cysteine, L-cystine, L-methionine, or D,L-homocysteine[1a] to the bacterial growth media.



14 1S, 2R



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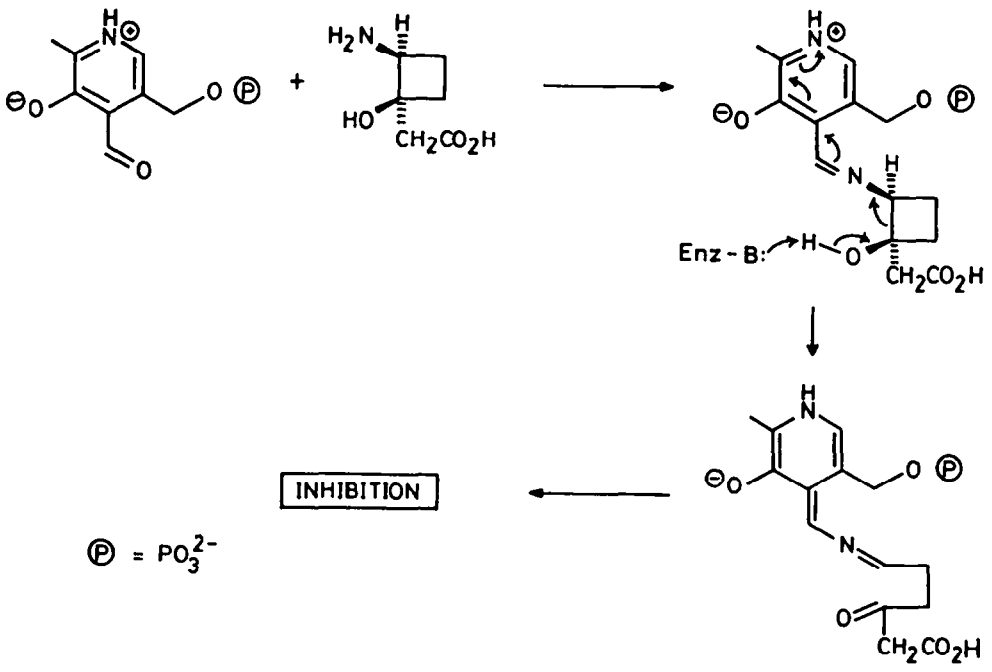
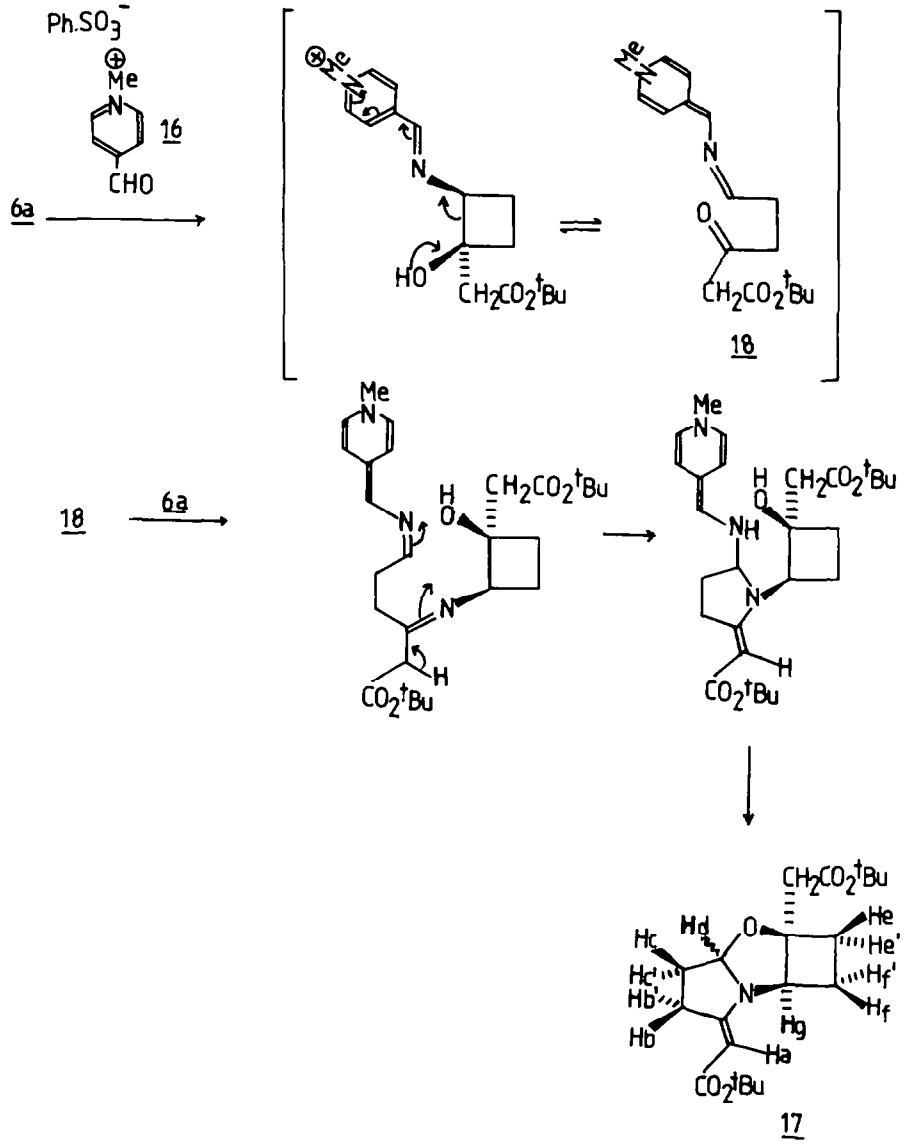


Scheme 3

The chemical feasability of such a cyclobutanol ring opening reaction process was investigated via a biomimetic study. Thus treatment of the racemic amine (6a) with 4-formyl-1-methylpyridinium benzenesulphonate (16)[9] (a chemical mimic for pyridoxal) gave the tricyclic 3-azaoxolane (17) whose structure was consistent with its ^1H and Jeener n.m.r. spectra, n.O.e. observations, U.V. spectra and accurate mass measurement (M^+ Found 365·2201. $C_{20}H_{31}NO_5$ requires 365·2202.). This unusual tricyclic product was presumably formed via the ring opened ketone (18), itself formed by a favoured cyclobutanol ring opening process (Scheme 4).



Scheme 4

We wish to thank the Department of Organic and Biological Chemistry of the University of Messina for financial support (to M.F.P.).

GENERAL EXPERIMENTAL

Reaction temperatures were recorded in degrees Celsius (°C). Reaction times were recorded in seconds (s), minutes (min.), or hours (h). Reactions were studied by T.L.C., I.R. or N.M.R. prior to work up. U.V. inactive compounds on T.L.C. were visualised with dodeca- molybdophosphoric acid (5% w/v in EtOH) followed by charring. Peptides and amino-acids were located on paper electrophoresis by using a cadmium ninhydrin reagent.[10] Reaction mixtures were evaporated at 40°C or below on a Buchi Rotavapor R110. Aqueous solutions were further freeze dried. Organic extracts were dried over magnesium sulphate. Both T.L.C. and P.L.C. were carried out on Merck Kieselgel 60 F_{254} and 60 PF_{254} plates; developing solvents are given in parenthesis. Melting points were determined on a Kofler hot stage or on a Buchi 510 capillary apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter. I.R. spectra were obtained as KBr discs or in CCl_4 solution unless otherwise stated, and were recorded on a 681 Perkin-Elmer spectrophotometer; only broad (br), medium (m), strong (s), and very strong (vs) bands were reported. U.V. spectra were recorded on a Perkin Elmer 555 UV-VIS spectrophotometer, solvent solutions are stated in parenthesis. ^1H N.M.R. spectra were recorded upon Brüker WH 300 MHz, AM 250 MHz, or AM 500 MHz NMR spectrometers using tetramethylsilane (organic solutions) and sodium-2,2,3,3-$^2H_4$-3-trimethylsilylpropionate (aqueous solutions) as internal standards unless otherwise stated. ^{13}C N.M.R. spectra were recorded upon a Brüker AM 250 or AM 500 NMR spectrometer using C^2HCl_3 = 77.00 p.p.m as internal reference for organic solutions or dioxan = 67.30 p.p.m as internal reference for aqueous solutions. Multiplicities were recorded as br (broad peak), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded upon ZAB IF or V. G. Analytical Ltd, 30F mass spectrometers [for ammonia desorption chemical ionisation (NH_3 D.C.I.) or positive argon fast atom bombardment (F.A.B.)] or V.G. Analytical Ltd 16F [for Electron Impact (E.I.)]. Microanalyses were recorded by Dr. F. B. Strauss, Microanalytical Laboratory, Dyson Perrins Laboratory, University of Oxford, or by the Microanalytical Laboratory, Department of Chemistry, University of Manchester. Preparative H.P.L.C. was carried out using two Gilson model 303 pumps, Rheodyne 7125 injector fitted with either 1 ml or 200 µl loops, and a Gilson model HM holochrome detector. The pumps were controlled from an Apple IIe computer using a Gilson Gradient Manager program. Zorbax octadecylsilane (ODS) (9.2 x 250 mm) or Zorbax octylsilane (C-8) (9.2 x 250 mm) Dupont pre-packed columns were used. Water:methanol or buffer:methanol (buffer ≡ 50 mM ammonium hydrogencarbonate solution) were used as H.P.L.C. eluants. The mobile phase flow rates (ml min^{-1}), solvent compositions and compound elution times are given in each experiment. Preparative paper electrophoresis at pH 1.8 was performed on Whatman No.1 filter-paper using a Locarte power pack at 4.0 Kilovolts for 1h. (70V cm^{-1}). pH 1.8 Electrophoresis buffer was prepared from water: acetic acid: formic acid (78:20:2). Ion-exchange resin purifications were performed on Bio-Rad AG 50WX-4 resin, 50-100 mesh, in the H^+ form which had been pre-equilibrated with pH 4.2, 0.2M sodium phosphate-citrate buffer.[11] After desalting with distilled water, compounds were eluted from the resin with a distilled water: pyridine (9:1) solution. All starting materials and reagents were purified and dried unless otherwise stated.

Microbiological Assay

Microbiological assays were performed by the "holed plate" assay method using the test organisms Staphylococcus aureus N.C.T.C. 6571, Bacillus subtilis A.T.C.C. 6633, and Escherichia coli ESS. The complex medium for the Staphylococcus aureus N.C.T.C. 6571 organism was prepared from Oxoid Lab Lemco (10.0g), bacterological peptone (10.0g), NaCl (5.0g), oxoid agar (10.0g) dissolved in distilled water (1000ml) and adjusted to pH 6.85-6.90 (2M sodium hydroxide). The cysteine free chemically defined media for the Bacillus subtilis A.T.C.C. 6633 and Escherichia coli ESS organisms used was prepared from sodium ammonium phosphate (1.5g), potassium dihydrogen phosphate (1.0g), magnesium sulphate (0.2g), glucose (5.0g), agar (11.0g), and asparagine (5.0g)dissolved in distilled water (1000ml) and adjusted to pH 6.8 (2M hydrochloric acid).

Preparation of (1R,2R)-and (1S,2S)-1-Hydroxy-2-[dibenzylamino]cyclobutane-1-acetic acid, t-butyl ester (4) and (1R,2S) and (1S,2R)-1-Hydroxy-2-[dibenzylamino]cyclobutane-1-acetic acid, t-butyl ester (5).

Zinc turnings (2.36g, 36.1mmol) activated by the method described by Vaughan et al.,[12] a small crystal of iodine and dry tetrahydrofuran (50ml) were stirred and heated at reflux under a nitrogen atmosphere. A solution of t-butylbromoacetate (5.96g, 30.6mmol) and α-dibenzylaminoketone (3) (5.31g, 20.0mmol) in tetrahydrofuran (50ml) was added dropwise over 30 min. (initially very slowly). The mixture was stirred and heated under reflux for a further 1h under anhydrous conditions. The solution was cooled, poured into 0.1M hydrochloric acid (150ml) and the pH adjusted to 2.0 with 2M hydrochloric acid. The excess zinc was filtered off, and the solution extracted with ethyl acetate (5 x 50ml). The organic layers were combined, washed with a saturated aqueous $NaHCO_3$ solution (3 x 50ml), water (3 x 50ml), dried, filtered and evaporated to yield a crude orange oil (6.954g). Purification by flash chromatography on Merck Kieselgel 60 [7 x 200g, eluant $CHCl_3$: diethyl ether (19:1)] gave (5) (0.57g, 7.5%) and (4) (4.76g, 62.5%).

For (5): Rf 0.57 [SiO_2, $CHCl_3$: diethyl ether (19:1)], m.p. 110-111°C (from acetone), (Found: C, 75.44; H, 8.14; N, 3.81. $C_{24}H_{31}NO_3$ requires C, 75.55; H, 8.19; N, 3.67%); $[\alpha]^2\beta$ 0° (c 0.44, EtOH); $\nu_{max.}$ (CCl_4) 3500 br (O-H), 1710 vs (C = O), 1395m - 1370s (CMe_3), 1155 vs (C-O), and 700 s (Ph-H) cm^{-1}; δH(300MHz, C^2HCl_3) 1.48(9H, s, ^tBu), 1.19-1.31 and 1.56-1.86 (4H, 2 x m, 3,4-CH_2), 2.69 and 2.85 [2H, ABq, (the upfield component of the AB quartet shows a long range coupling J 1.5Hz), J_{AB} 16.5Hz, $CH_2CO_2{}^tBu$], 3.20-3.26(1H, m, 2-CH), 3.37 and 3.70(4H, ABq, J_{AB} 14.5Hz, 2 x CH_2Ph), 4.22(1H, s, OH), and 7.20-7.33(10H, m, 2 x Ph-H); δC(62.85 MHz, C^2HCl_3) 19.95(t, cyclic-CH_2), 28.16(q, CMe_3), 29.74(t, cyclic-CH_2),

38.82(t, $CH_2CO_2{}^tBu$), 54.65(t, 2 x CH_2Ph), 66.8[d, C(2)], 75.7[s, C(1)], 81.77(s, CMe_3), 126.72, 128.02, and 129.03(3 x d, aryl-C), 138.26(s, aryl-C), and 173.28(s, $CO_2{}^tBu$) p.p.m.; m/e (NH_3 D.C.I.) 382(MH^+, 100%), 223(45), 132(30), and 91(46).

For (4): Rf 0.44 [SiO_2, $CHCl_3$: diethyl ether (19:1)], m.p. 64-5°C (from light petroleum 40-60°C), (Found C, 75.49; H, 8.05; N, 3.79. $C_{24}H_{31}NO$ requires C, 75.55; H, 8.19; N, 3.67%); $[\alpha]^2_D$ 0° (c 0.9, EtOH); ν_{max}.(CCl_4) 3380 br (O-H), 1730 vs (C = O), 1395 m - 1370 s (CMe_3), 1150s (C-O), and 700 s (Ph-H) cm^{-1}; δH(300MHz, C^2HCl_3) 1.44(9H, s, ^tBu), 1.90-1.99 and 2.02-2.17(4H, 2 x m, 3,4-CH_2), 2.38 and 2.53(2H, ABq, J_{AB} 14.5Hz, $CH_2CO_2{}^tBu$), 3.25(1H, t, J 8.0Hz, 2-CH), 3.57-3.65(4H, ABq, J_{AB} 14.5Hz, 2 x CH_2Ph), 4.54(1H, br s, OH), and 7.22-7.34(10H, 2 x Ph-H); δC(62.85 MHz, C^2HCl_3) 22.71(t, cyclic-CH_2), 28.13(q, CMe_3), 28.88(t, cyclic-CH_2) 47.12(t, $CH_2CO_2{}^tBu$), 53.70(t, 2 x CH_2Ph), 63.09[d, C(2)], 74.09[s, C(1)], 80.62(s, CMe_3), 127.08, 128.25 and 129.07(3 x d, aryl-C), 137.66(s, aryl-C) and 170.23(s, $CO_2{}^tBu$) p.p.m.; m/e (NH_3 D.C.I.) 382(MH^+, 100%), 223(55), 132(43), and 91(25).

Preparation of (1R,2R)- and (1S,2S)-1-Hydroxy-2-aminocyclobutane-1-acetic acid, t-butyl ester (6a).

A suspension of 10% Palladium on carbon (150mg) and (4) (763mg, 2.0mmol) in methanol (20 ml, O_2 free) was hydrogenated at atmospheric pressure at 27-30°. After 10 and 20h two fresh suspensions of the catalyst (50mg) in methanol (5ml) were added. The mixture was stirred for a total of 24h during which time hydrogen (103ml, 4.2mmol) was consumed. The catalyst was removed by filtration through celite and the methanolic filtrate was evaporated under reduced pressure to yield (6a) as a crude solid (360mg), m.p. 68-9°C (from ethyl acetate). (Found: C, 59.90; H, 9.14; N, 6.94. $C_{16}H_{31}NO_3$ requires C, 59.68; H, 9.51; N, 6.96%); $[\alpha]^2_D$ 0° (c 0.5, EtOH); ν_{max} (CCl_4) 3490-3220 br (O-H,N-H), 1730 vs (C=O), 1395 m - 1370 s (CMe_3), and 1155 vs (C-O) cm^{-1}; δH (300 MHz, C^2HCl_3) 1.46 (9H, s, ^tBu), 1.64-1.88 and 2.11-2.20(4H, 2 x m, 3,4-CH_2), 2.43 and 2.55(2H, ABq, J_{AB} 15.5Hz, $CH_2CO_2{}^tBu$), and 3.34-3.39(1H, m, 2-CH), δC(62.85 MHz, C^2HCl_3), 26.09(t, cyclic-CH_2), 28.16(q, CMe_3), 30.05(t, cyclic-CH_2), 45.48(t, $CH_2CO_2{}^tBu$), 53.66[d, C(2)], 74.36[s, C(1)], 81.06(s, CMe_3), and 171.48(s, $CO_2{}^tBu$) p.p.m.; m/e (NH_3 D.C.I.) 202(MH^+, 100%), 146 (32), and 43 (13).

Preparation of (1R,2S)- and (1S,2R)-1-Hydroxy-2-aminocyclobutane-1-acetic acid, t-butyl ester (6b).

A suspension of 10% Palladium on carbon (150mg) and (5) (763mg, 2.0mmol) in ethanol (20ml, O_2 free) was hydrogenated at atmospheric pressure at 27-30°. After 10 and 20h two fresh suspensions of catalyst (50mg) in EtOH (5ml) were added. The mixture was stirred for a total of 24h during which time hydrogen (104ml, 4.26mmol) were consumed. The catalyst was filtered off through celite and the filtrate evaporated to yield crude (6b) (355mg); m.p. 85-6°C (from ethyl acetate) , $[\alpha]^2_D$ 0° (c 0.5, EtOH); ν_{max} (CCl_4) 3500 br (O-H), 3400 sh (NH), 1710 vs (C=O), 1395-1370 s (CMe_3), and 1155 vs (C-O) cm^{-1}; δH(250 MHz, C^2HCl_3), 1.49(9H, s, ^tBu),1.14-1.26, 1.72-1.79 and 2.02-2.14(4H, 3 x m, 3,4-CH_2), 2.44 and 2.57(2H, ABq, J_{AB} 16Hz, $CH_2CO_2{}^tBu$), and 3.45(1H, t, J 9Hz, 2-CH); δC(62.85MHz, C^2HCl_3) 22.72(t, cyclic-CH_2), 28.09(q, CMe_3), 29.01(t, cyclic-CH_2), 38.56(t, $CH_2CO_2{}^tBu$), 58.01 [d, C(2)], 77.17 [s, C(1)], 81.71 (s, CMe_3), and 173.15 (s, $CO_2{}^tBu$) p.p.m.; m/e (E.I.) 202 (MH^+, 5%), 184 (3), 57 (46), and 43 (100); [(E.I.) Found 201.1365. $C_{10}H_{19}NO_3$ requires 201.1364]

Preparation of (1R,2R)- and (1S,2S)-1-Hydroxy-2-aminocyclobutane-1-acetic acid (7).

Crude (6a) [180mg, from (4)(1.0mmol)] was dissolved in trifluoroacetic acid/anisole (9:1, 1.5ml) and the solution stirred at 20-25°C for 30 min. The solution was evaporated, dissolved in water (20ml), then washed with diethylether (3 x 30 ml). The aqueous phase was evaporated, then dissolved in pH 4.2, 0.2M sodium phosphate-citrate buffer (5ml) and desalted by ion-exchange chromatography [resin wet volume = 10ml, washed with water (500ml), eluted with water: pyridine (9:1) (500ml)]. Evaporation of the aqueous pyridine layer gave crude (7) (116mg) which was further purified by H.P.L.C. on a Zorbax ODS column, using pH 7.5, 50mM ammonium hydrogencarbonate as the mobile phase. Typically crude (7) [10mg in water (1ml)] was injected using a mobile phase flow rate of 4ml min^{-1}, with U.V. detection set at 220 nm.(7) eluted from the column after 345 s. Repeated H.P.L.C. runs gave (7) [109mg, 75% from (4)], m.p. 173-181°C (sealed tube) (from H_2O:EtOH), (Found: C, 49.21; H, 7.48; N 9.37. $C_6H_{11}NO_3$ requires C, 49.64; H, 7.63; N, 9.65); $[\alpha]^2_D$ 0° (c 0.26, H_2O); νmax. (KBr) 3420 br (O-H), 2960 br (CO_2H), 2640-2150 sh ($^+NH_3$),1565 vs-1400 s (CO_2^-), and 1280 s (O-H) cm^{-1}; δ_H (500MHz, ^2H_2O) 2.05-2.16, and 2.22-2.31 (4H, 2 x m, 3,4-CH_2), 2.57 and 2.61 (2H, ABq, J_{AB} 16.0Hz, CH_2CO_2H), and 3.75 (1H, m, 2-CH); δC (62.85 MHz, ^2H_2O) 20.81 (t, cyclic-CH_2), 22.92 (t,cyclic-CH_2), 45.72 (t,CH_2CO_2H), 51.27 [d,C(2)], 73.62 [s,C(1)], and 178.74 (s, CO_2H) p.p.m.; m/e (F.A.B.) 146 (MH^+, 100%),69 (12), and 43 (13).

Preparation of (1R,2S)- and (1S,2R)-1-Hydroxy-2-aminocyclobutane-1-acetic acid (8).

Crude (6b) [178mg, from (5) (1.0mmol)] was dissolved in trifluoroacetic acid/anisole (9:1, 1.5ml) and then treated as in the preparation of (7) from (6a), to yield crude (8) (120mg). Final purification by H.P.L.C. [as for (7) from (6a)] gave (8) [113mg, 78% from (5)], H.P.L.C. retention time 330 s, m.p. 195-204 °C (sealed tube) (from H_2O:EtOH), (Found: C, 49.45; H, 7.70; N, 9.70. $C_6H_{11}NO_3$ requires C, 49.64; H, 7.63; N, 9.65%); $[\alpha]^2_D$ 0° (c 0.33, H_2O); νmax. (KBr) 3400 br (O-H), 2960 br (CO_2H), 2640 br and 2160 br ($^+NH_3$), 1545-1400 vs (CO_2^-), and 1280 s (O-H) cm^{-1}; δH (500MH_Z,^2H_2O) 1.68-1.76, 1.93-2.00 and 2.11-2.24 (4H,3 x m,3,4-CH_2), 2.65 and 2.77 (2H, ABq, J_{AB} 15Hz, CH_2CO_2H), and 3.79 (1H,t,J 9Hz, 2-CH); δC (62.85 MHz, ^2H_2O) 18.57 (t,cyclic-CH_2), 30.87 (t, cyclic-CH_2), 43.13 (t, CH_2CO_2H), 55.06 [d, C(2)], 74.95 [s, C(1)], and 179.10 (s,CO_2H) p.p.m.; m/e (NH, D.C.I.) 146 (MH^+, 100%), 128 (31), and 43 (79).

Preparation of (1S,2S)-1-Hydroxy-2-[N-t-Butyloxycarbonyl-(S)-valylamino] cyclobutane-1-acetic acid, t-butyl ester (9) and (1R,2R)-1-Hydroxy-2-[N-t-butyloxycarbonyl-(S)-valylamino] cyclobutane-1-acetic acid,t-butyl ester (10).

To a stirred solution of N-t-butoxycarbonyl-(S)-valine[13] (435mg, 2.0mmol) and 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (544mg, 2.2mmol) in dichloromethane (30ml) was added dropwise a solution of crude (6a) [360mg, from (4)(2.0mmol)] in dichloromethane (20ml) over 15 min. The reaction was stirred under argon at 20-25°C for 24h, washed with 2M hydrochloric acid (3x50ml), saturated aqueous $NaHCO_3$ solution (3x50ml), water (2x50ml), dried, filtered, and evaporated to yield crude (9)+(10) (672mg). Purification by p.l.c. [6x 40x20x0.1cm plates, EtOAC: hexane (2:1)] gave a 1:1 mixture of (9)+(10) (556mg, 69%). (9) Could be obtained pure by fractional

recrystallization from diethylether. Alternatively (9) and (10) could be separated by H.P.L.C. [column: Zorbax C-8; mobile phase: MeOH: H_2O (65:35); detection: 220nm, flow rate: 4ml. min^{-1}; retention time (9): 31 min, 50s,(10): 35 min, 20s.]

For (9): m.p. 165-166°C (from diethyl ether), (Found: C, 59.98; H 9.26; N, 7.01. $C_{20}H_{36}N_2O_6$ requires C, 59.98; H 9.06; N, 6.99%); $[\alpha]_D^{\circ}$ - 22.8° (c 0.42, EtOH); νmax. (CCl_4) 3440m (N-H), 1730 vs (C=O), 1680 vs (CONH), 1490 s (N-H), 1395 m - 1370 s (CMe_3),1240vs-1215vs (C-O)cm^{-1}; δ_H (250 MHz, C^2HCl_3) 0.92 and 0.97 [6H,2 x d, J 7Hz, $CHMe_2$), 1.45 (18H, s, 2 x ^tBu), 1.81 - 2.30 (5H, m, 3,4-CH_2,$CHMe_2$), 2.53 and 2.57 (2H,ABq, J_{AB} 16.5 Hz $CH_2CO_2{}^tBu$), 3.89-3.94 (1H,m, $CHCHMe_2$), 4.16-4.25 (2H,m,OH,2-CH), 5.07-5.10 (1H,br.d,NH), and 6.50-6.53 (1H,br.d,NH),δC (125.76 MHz,d_6-acetone) 18.01 and 19.78 (2 x q, $CHMe_2$), 25.48 (t,cyclic-CH_2), 28.30 and 28.58 (2 x q, 2 x CMe_3), 30.86 (t,cyclic-CH_2), 31.78 (d, $CHMe_2$), 46.09 (t,$CH_2CO_2{}^tBu$), 51.55 [d,C(2)], 60.54 (d,NHCHCO),77.46 [s, C(1)], 79.14 and 81.07 (2 x s, 2 x CMe_3), 156.6 (s, $NHCO_2{}^tBu$), 171.38 and 171.69 (2 x s, NHCO, CO_2) p.p.m.; m/e (NH_3 D.C.I.) 401 (MH^+, 100%), 345 (17), 289 (12), and 72 (9).

For(10): m.p. 121-122°C [from light petroleum (b.p. 40-60°C): EtOAC (9:1)], (Found: C, 60.20; H, 9.30; N, 6.70. $C_{20}H_{36}N_2O_6$ requires C, 59.98; H, 9.06; N, 6.99%); $[\alpha]_D^{\circ}$ + 20.0° (c 0.53, EtOH); νmax (CCl_4) 3435 m (N-H), 1715 vs (C=O), 1680 s (CONH), 1490 s (N-H), 1395m-1370s (CMe_3),and 1230 m (C-O) cm^{-1}; δH (300 MHz, C^2HCl_3) 0.92 and 0.96 (6H, 2 x d, J 7Hz, $CHMe_2$), 1.44 and 1.45 (18H, 2 x s, 2 x ^tBu), 1.81-2.29 (5H,m, 3,4-CH_2, $CHMe_2$), 2.52 and 2.56 (2H,ABq, J_{AB} 16.5Hz, $CH_2CO_2{}^tBu$),3.85-3.90 (1H,m,$CHCHMe_2$),4.13-4.25 (2H,m, OH, 2-CH), 5.02-5.05 (1H,br. d, NH), and 6.40-6.51 (1H,br.d.,NH); δC (125.76MHz, d_6-acetone) 18.25 and 19.71 (2 x q, $CHMe_2$), 25.19 (t,cylic-CH_2), 28.32 and 28.60 (2 x q, 2 x CMe_3), 30.54 (t,cyclic-CH_2), 31.61 (d, $CHMe_2$), 46.40 (t, $CH_2CO_2{}^tBu$), 51.62 [d, C(2)],60.86 (d,NHCHCO), 77.72 [s, C(1)], 79.26 and 80.84 (2 x s, 2 x CMe_3), 156.71 (s, $NHCO_2{}^tBu$), 171.20 and 171.89 (2 x s, NHCO, CO_2) p.p.m.; m/e (NH_3 D.C.I.) 401 (MH^+, 100%), 345 (60), 289 (53), 143 (34), and 72 (54).

Preparation of (1S,2S)-1-Hydroxy-2-[(S)-valylamino]cyclobutane-1-acetic acid (1) and (1R,2R)-1-Hydroxy-2-[(S)-valylamino]cyclobutane-1-acetic acid (11).

The diastereoisomeric mixture (9)+(10) [400mg, 1.0mmol (9):(10) = 1:1] was dissolved in trifluoroacetic acid/anisole (9:1, 2ml) and stirred at 20-25°C for 30 min. The solvent was evaporated, the residue dissolved in water (30ml) and washed with diethyl ether (3x30ml). The aqueous phase was evaporated, then dissolved in pH 4.2, 0.2M sodium phosphate-citrate buffer (10ml) and then desalted by ion-exchange chromatography [resin wet volume=10ml, washed with water (500ml), eluted with water: pyridine (9:1)(500ml)]. Evaporation of the aqueous pyridine layer gave crude (1) + (11) (241mg, 99%) which were separated by H.P.L.C. on a Zorbax ODS column using 50mM pH 7.5 ammonium hydrogencarbonate: methanol (4:1) as the mobile phase. Typically crude (1) + (11) [10mg in water (1ml)] was injected using a flow rate of 4ml min^{-1}, with U.V. detection set at 220 nm. (1) eluted from the column after 5 min., 25s and (11) eluted after 10 min., 20s. Repeated H.P.L.C. runs gave (1)(115mg, 94%) and (11) (110mg, 90%).

For (1): m.p. 239-246°C (dec.; sealed tube) (from H_2O:EtOH) (Lit.,[1a] 247-250°C), (Found: C,53.9; H,8.25 ; N,11.1. $C_{11}H_{20}N_2O_4$ requires C, 54.08; H, 8.25; N, 11.47%); $[\alpha]_D^{\circ}$ + 6.3° (c 1.7, H_2O) (Lit.,[1a] 8.4° (c 1, H_2O)[e]; νmax. (KBr) 3370 s - 3260 br (O-H, N-H),2970 br (O-H), 2560-2150 br (N-H), 1670 vs (CONH), 1520 vs (N-H), 1620 s-1400 s (CO_2), and 1280 s (O-H) cm^{-1}; δH (500MHz, ^2H_2O) 1.04 and 1.05 (6H, 2 x d, J 7Hz, $CHMe_2$), 1.92-2.03 and 2.18-2.27 (5H,2 x m, 3,4-CH_2 and $CHMe_2$), 2.50 and 2.52 (2H,ABq, J_{AB} 15Hz, CH_2CO_2), 3.79 (1H,d,J 6Hz,NCHCO) and 4.21 (1H,m, 2-CH); δC (62.85 MHz, ^2H_2O) 16.80 and 17.77 (2 x q, 2 x Me), 23.06 (t, cyclic-CH_2), 29.75 (t, cyclic-CH_2),29.95 (d, $CHMe_2$), 45.86 (t, CH_2CO_2), 51.67 and 58.80 [2 x d, C(2) and NHCHCO], 76.38 [s, C(1)], 169.25 (s, CONH), and 179.16 (s, CO_2) p.p.m.; m/e (F.A.B.) 245 (MH^+, 100%), 146 (42), and 72 (88). The sample was identical by ^1H and ^{13}C n.m.r. to the authentic sample [e] (individual and mixed experiments)

For(11): m.p. 201-210°C (dec., sealed tube) (from EtOH), (Found: C, 53.97;H, 8.26; N, 11.27. $C_{11}H_{20}N_2O_4$ requires C, 54.08; H, 8.25; N, 11.47%); $[\alpha]_D^{\circ}$ + 81° (c 1.7, H_2O); νmax. (KBr) 3415 s (NH), 3100 br (NH), 2960 s (O-H), 2540- 2145 br (N-H), 1685 vs (CONH), 1505 vs (N-H) 1620 vs - 1400 vs (CO_2), and 1270 s (O-H) cm^{-1}; δH (500MHz, ^2H_2O) 1.016 and 1.020 (6H, 2 x d, J 7Hz, $CHMe_2$), 1.91-2.03 and 2.13-2.26 (5H, 2 x m, 3,4-CH_2 and $CHMe_2$), 2.51-2.53 (2H, ABq, J_{AB} 15.5Hz, CH_2CO_2), 3.72 (1H, d, J 6Hz, NCHCO), and 4.22 (1H,m, 2-CH); δC (62.85 MHz, ^2H_2O) 17.72 and 18.40 (2 x q, 2 x Me), 23.95 (t, cyclic-CH_2), 30.23 (t,cyclic-CH_2) 30.59 (d,$CHMe_2$), 46.77 (t, CH_2CO_2), 52.07 and 59.41 [2 x d, C(2) and NHCHCO], 77.62 [s, C(1)], 169.47 (s, CONH), and 180.13 (s, CO_2) p.p.m.; m/e (F.A.B.) 245 (MH^+, 100%), 146 (40), and 72 (99).

Preparation of (1S,2S)-1-Hydroxy-2-aminocyclobutane-1-acetic acid (2).

The dipeptide (1)(200mg, 0.82 mmol.) was dissolved in hydrochloric acid (2M, 10ml.), and the mixture refluxed for 10 h. The solution was evaporated, the residue dissolved in water (20ml), washed with diethyl ether (3x 30ml), and freeze dried to give a crude residue (255mg.). Direct ^1H N.M.R. calibration analysis at 500MHz on the crude hydrolysis product indicated 18% yield of (2) and the presence of unreacted (1) (33%) prior to work-up. Purification by preparative electrophoresis at pH 1.8 (8 sheets of 40 x 57cm Whatman No.1 paper) gave, upon extraction of the ninhydrin active band that moved 27-35 cm towards the cathode with 0.01N hydrochloric acid, crude (2) (61mg), free from valine. Final purification was achieved by H.P.L.C. on a Zorbax ODS column using 50mm pH 7.5 NH_4HCO_3 solution as the mobile phase with detection set at 220 nm. Thus crude (2) [10 mg in water (1ml)] was injected using a mobile flow rate of 2ml min^{-1}, and (2) eluted after 630 s. Repeated H.P.L.C. runs gave (2) [12 mg, 10%], $[\alpha]_D^{\circ}$ +8.4° (c 0.13 in H_2O), ^1H n.m.r (500MHz) as for the racemate (7).

Preparation of (1R,2R)-1-Hydroxy-2-aminocyclobutane-1-acetic acid (13).

An identical procedure (except that the mixture was refluxed for 20h.), to that used for the preparation of (2) was employed. Thus (11) (100 mg, 0.41 mmol) gave upon hydrolysis and purification, (13) (12 mg, 20%), $[\alpha]_D^{\circ}$ -7.8 (c 0.11, H_2O), ^1H n.m.r. (500MHz) as for the racemate (7). Direct ^1H N.M.R. analysis at 500 MHz on the crude hydrolysis product indicated a 36% yield of (13) and the presence of unreacted (11) (11%) prior to work-up.

Preparation of the 3-azaoxolane (17).

To a stirred solution of crude (6a) [168mg, from(4) (1.0mmol)] in dichloromethane: dimethylformamide (3:1) (40ml) at 20-25°C was added 4-formyl-1-methylpyridiniumbenzenesulphonate[9] (140 mg, 0.5mmol) in one portion. The mixture was stirred for 45 min., evaporated, the residue dissolved in dichloromethane (50ml), washed with water (3x50ml), dried, filtered and evaporated. The resultant oil (132mg) was purified by p.l.c. [3x 20x20x0.1 cm SiO_2 plates, hexane: ethylacetate (2:1)] to yield (17) (18mg, 10%), t.l.c. [SiO_2, hexane: ethyl acetate (2:1) Rf 0.46; $[\alpha]_D^{\circ}$ 0° (c 0.27, EtOH); λmax (EtOH) 272nm (ε $1.4x10^3$); νmax. ($CHCl_3$) 1715 vs (C=O), 1395 m - 1370 s (CMe_3), and 1135 s (C-O) cm^{-1}; δH (300MHz, C^2HCl_3) 1.44 and 1.45 (18H, 2 x s, 2 x ^tBu), 1.80-1.91, 1.97-2.08, and 2.18-2.36 (6H, 3 x m, H_c,H_c',H_e,H_e',H_f,H_f'), 2.62 (2H,s, $\underline{CH_2}CO_2{}^tBu$), 3.00-3.12 and 3.34-3.46 (2H, 2 x m, H_b,H_b'), 4.21-4.23 (1H,m, H_g), 4.79 (1H, s, -CH), and 5.53-5.56 (1H,m,H_d); m/e (NH_3 D.C.I.) 366 (MH^+, 100%),310(70),292(34),254(23),208(28),166 (51),152(28),and 108(26),- [(E.I.) Found 365.2201. $C_{20}H_{31}NO_5$. requires 365.2202].

The structure as (17) was consistent with a Jeener n.m.r. experiment[10] Nucleur Overhauser difference spectra were recorded on (17) in deuteriochloroform solution using a saturation time of 5 s, with R.F. field strengths of 5-15Hz and with a pulse width of 90°. The values of the n.O.e enhancements upon irradiation of H_a, H_g, and $\underline{CH_2}CO_2{}^tBu$ are listed below.

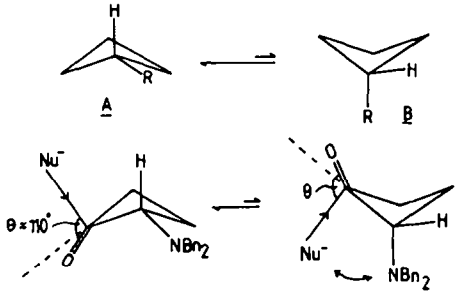
Proton irradiated	Proton enhanced	n.O.e value (%)
H_a	H_g	20.5
H_g	$\underline{CH_2}CO_2{}^tBu$	2.1
	Ha	24.5
$\underline{CH_2}CO_2{}^tBu$	Hg	2.3

These observations are consistent with the E- vinylogous urethane geometry. However due to the overlap of H_c,H_c',H_e,H_e',H_f,and H_f' in the ^1H n.m.r spectrum it was not possible to deduce the stereochemistry of H_d by n.O.e experiments.

Footnotes

† A preliminary report of this work has been reported in communication form, see R.M. Adlington, J.E. Baldwin, R.H. Jones, J.A. Murphy, and M.F. Parisi, J. Chem. Soc., Chem. Commun., 1983, 1479.

† Of the two conformations of a substitued cyclobutane it is the one in which the substituent (R) is equatorial that is favoured (eg A) due to non-bonded repulsions in the axial conformation (eg B)[3]. This coupled with the known tendency for the carbonyl function of a cyclobutanone to be distorted towards the inner endo face [4a,b] suggests that a favoured nucleophilic attack at the requisite angle of attack (θ ≈110°)[5] will occur from the exo-face to provide a trans - selective addition.



" Trans"- addition > "Cis"- addition

A similar trans - selective addition has been observed in the lithium aluminium hydride reduction of a substituted cyclobutanone[6a], and in the (1-phenylvinyl)lithium addition to a substituted cyclobutanone [6b].

‡ The deprotected forms (1) and (11) were more suitable for separation by reverse phase H.P.L.C. than their protected forms (9) and (10).

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8. We thank Dr. J.P. Scannell (Hoffman-La Roche Inc., Nutley, New Jersey, 07110, U.S.A.) for an authentic sample of (1). We measured the optical rotation for the authentic sample of (1) from Scannell and found it to be in better agreement with our value, $[\alpha]_D^{\circ}$ + 6.2° (c 0.29, H_2O).

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